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(54) Title: ION CHANNEL SENSOR TYPING

(57) Abstract

An ion channel sensor determines the compatibility of blood products from two samples. Blood products are introduced into a biosensor cell that comprises an ICS in which the ionophore and membrane spanning lipids (MSL's) present molecules capable of binding to epitopic sites on a red blood cell such that the arrival of the blood cell at the membrane crosslinks the ionophore and MSL and affects the ionic current through the membrane. The blood products must pass a thin layer of percolation beads to reach the membrane. The preferred molecules capable of binding to epitopic sites on a red blood cell are antibodies that selectively bind to glycoprotein molecules that are present on the surface of the red blood cell. In a further embodiment of the present invention the presence of antibodies to multiple epitopes on a large antigen is detected by anti-Fc Fab' being attached to both the ion channel and the membrane spanning lipid within the sensor membrane. The presence of Abs to a variety of epitopic sites on large protein Ags is detected by tethering the large Ag or Ag fragment to both the MSL and to the mobile outer layer ion channels. The determination of the class of an Ab, for example as to whether it's IgG or IgM is determined by having the Abs pass through a bead bed labelled with either IgG or IgM molecules preventing the passage of bound antibody to the electrode surface and to the reporter antigens. The invention further provides for the determination of the coagulation state of a population of erythrocytes by using an array of small diameter (typically 1-100 μ, preferably 10-90μ) gold electrodes bearing a biosensor membrane in which the Fab' recognition element was typically anti-glycoprotein. Each electrode is independently addressable using a multiplexed array.

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Ion Channel Sensor Typing

Field of the invention

This invention relates to serologic methods and apparatus comprising a membrane biosensor for determining blood type and for identifying antibodies to epitopic sites on blood molecules or large antigens. Further, it relates to the use of a membrane biosensor to determine the immunoglobin class of an antibody.

Background of the invention

In order to utilise stored blood or blood components for transfusion it is necessary to test for compatibility with blood type of the recipient. Blood typing is a routine procedure for all blood bank blood and components such as plasma or serum. It is generally understood which blood types are compatible and which will result in clotting that prevents effective transfusion. Indeed the infusion of an incorrect blood type into a recipient is potentially fatal. Therefore procedures have been developed to test a sample's blood cells against a patient's serum or vice versa. The procedure consists of mixing the two and observing the formation or absence of coagulation.

In a typical procedure glass tubes impregnated with a filtering material are contacted at one end with the sample/patient mixture and the penetration of the mixture through the filter is observed. In order to quicken the process the tube is centrifuged. Inspection of the filter material through the wall of the tube is thus indicative of the extent of coagulation and thus of the incompatibility of the sample and the patient's blood types.

Procedure have also been developed to detect the presence of antibodies to multiple epitopes on a large antigen such as a viral particle, a protein fragment from a cell or a virus or an intact cell. A related procedure has been developed to detect the presence of Abs to a variety of epitope sites on large protein Ags. The prior art method employs deposition of the virus protein fragment or cell (the antigen) onto the surface of an ELISA cell. The patient serum containing the antibodies to a range of eptiopic sites is added. Following incubation to allow the binding of Ab to these epitopes the well volume is washed of free antibody and a reporter anti-Fc antibody tagged with an enzyme label is added and binds to the sample Abs which remain

attached to the large Ag, by which means the presence of the bound Ab on the Ag is detected.

It is often of clinical interest to determine the species of Ab, as to whether it is IgG or IgM. The prior art uses the method just described except that the tagged reporter Ab is either anti-IgG or anti-IgM.

Furthermore it is desirable to determine the coagulation state of a population of erythrocytes. Currently this is achieved by means of visual inspection either by a thin film of blood on a reflecting background or via the percolation properties under the action of a centrifugal force.

Biosensors have been constructed comprising biomembranes which are a double layer of closely packed amphiphilic lipid molecules. The molecules of these bilayers exhibit the random motions characteristic of the liquid phase, in which the hydrogen tails of the lipid molecules have sufficient mobility to provide a soft, flexible, viscid surface. The molecules can also diffuse sideways freely within their own monolayer so that two neighbouring lipids in the same monolayer exchange places with each other about once every microsecond, while the lipid molecules in opposite monolayers exchange places on the average of one a year.

These membranes may incorporate a class of molecules, called ionophores, which facilitate the transport of ions across these membranes. Ion channels are a particular form of ionophore, which as the term implies, are channels through which ions may pass through membranes. A favoured ionophore is gramicidin A which forms aqueous channels in the membrane. Examples of such biosensors are disclosed in the following Internationa Patent Applications, the disclosures of which are incorporated herein by cross reference:

PCT/AU88/00273, PCT/AU89/00352, PCT/AU90/00025, PCT/AU92/00132, PCT/AU93/00509, PCT/AU93/00620, PCT/AU94/00202, PCT/AU95/00763, PCT/AU96/00304, PCT/AU96/00368, PCT/AU96/00369 and PCT/AU96/00482.

The first of these references discloses receptor molecules conjugated with a support that is remote from the receptor site. The support may be a lipid head group, a hydrocarbon chain, a cross-linkable molecule or a membrane protein.

The inner level of the membrane may be adjacent a solid surface with groups reactive with the solid surface, and spaced from the surface to provide a reservoir region as disclosed in U.S. Patent No. 5,401,378.

Biosensors based on ion channels or ionophores contained within lipid membranes tethered to or deposited onto metal electrodes are disclosed in Australian Patent 623,747 and U.S. patent 5,234,566. Those references disclose a membrane bilayer in which each layer has incorporated therein ionophores and in which the conductance of the membrane is dependent upon the presence of absence of an analyte. The disclosure of Australian Patent 623,747 (incorporated herein by reference) describes various ionophore gating mechanisms termed local disruption gating, extended disruption gating, vertical disruption gating, and extended displacement gating mechanisms to modify the conductivity of the membrane in response to the presence of an analyte. In each of those gating mechanisms an inner layer of the membrane (the layer closer to the solid electrode surface, if any) contains immobilised or tethered half membrane spanning ion channels which an outer layer contains more mobile half membrane spanning ion channels. One method for immobilising the ion channels of the inner layer is to employ a polymerisable lipid layer and then cross-link the molecules of the inner monolayer and the ionophore. The conductivity of the membrane is altered by the extent to which opposing half membrane spanning ion channels align to establish a membrane spanning channel for ion transmission across the membrane.

In local disruption gating receptor molecules are linked to mobile ionophores is the outer layer that are aligned with tethered or immobilised ionophores in the inner layer. The introduction of an analyte particle that binds to two adjacent receptors in the outer layer causes the disruption of the orderly alignment of the membrane spanning ionophore. In the case of local disruption gating a loss of conductivity occurs due to the deformation of the ionophores of the outer layer caused by the bonding of the analyte with the adjacent receptors.

The mechanism of extended disruption gating is similar, except that the displacement of the mobile ionophore is greater. In extended disruption gating the binding of pairs of receptors to the same analyte particle cause the outer layer ionophores to move completely out of alignment with the inner layer ionophores.

The mechanism of vertical disruption gating is also similar. In that case the presence of the analyte particle bound to two receptor molecules causes a separation of the two layers that disrupts the continuity of the ion channel across the membrane.

The mechanism of extended displacement gating utilises two different receptors that bind to each other and are linked receptively to a half membrane ionophore and a membrane molecule. The binding of these two receptor molecules to each other displaces the ionophore and disrupts conductivity. The analyte competes with the second receptor for the binding site on the first receptor. The presence of the analyte breaks the bond between the two receptors and allows the half membrane ionophores to realign and provide an ion conductive path. Each of these mechanisms has in common that the binding of the analyte to the receptor molecule causes a change in the relationship between two half membrane spanning monomers such that the flow of ions across the membrane via the ionophores is allowed or prevented.

Brief Description of the Invention

The present invention employs in one preferred embodiment an ion channel sensor to determine the compatibility of blood products from two samples. It has the advantage of simplifying and speeding the determination of compatibility of the samples. In a preferred method of the present invention red blood cells from one sample together with plasma from a second sample comprise an analyte that is introduced into a specialised biosensor cell.

Accordingly in one aspect the present invention consists in a biosensor for detecting the coagulation of blood in an analyte comprising

a container for a blood sample subject to testing, said container having

a volume for holding a sample of blood products subject to testing,

a first electrode in contact with said sample,

a second electrode below said volume,

means responsive to the change of conduction between said electrodes,

a membrane below said volume, said membrane located between said electrodes, and having ion channels therein covalently attached to detector

molecules at an upper surface that bind to epitopic sites on blood components, said ion channels having a conductivity to ions that changes upon the binding of said molecules,

said volume contacting a filter layer between said membrane and said volume, wherein said blood components if uncoagulated pass said filter and contact said membrane.

In a preferred embodiment the membrane further comprises membrane spanning components attached to a second species of molecules that bind to sites on blood components, wherein the presence of a blood component at the surface of the membrane crosslinks the ion channels to said second species of molecules. It is further preferred that the second species of molecules is the same as said detector molecules.

The detector molecules and second species of molecules preferably bind to epitopic sites on a blood component, and are preferably antibodies to glycophorin.

In a further preferred embodiment the membrane spanning components are tethered by linking and spacing molecules to said second electrode.

The change in conductivity upon the binding to the membrane may be as a result of any gating mechanism such as local disruption gating, extended disruption gating, vertical disruption gating or extended displacement gating.

Examples of material which can be used in the filter layer are wellknown to persons skilled in the field and include the material used "BioVue" ™ sold by Ortho Diagnostic Systems Inc.

In a second aspect the present invention consists in a method of determining compatibility of two blood samples, the method comprising the steps of:

(1) mixing two blood samples to form a blood mix;

(2) introducing the blood mix into the biosensor of the first aspect of the present invention; and

(3) measuring change in conductance between the two electrodes of the biosensor.

In a third aspect the present invention consists in a method of screening for the presence of antibodies or antigens of interest in a biological sample, the method comprising the steps of:

(1) introducing the biological sample into a biosensor, the biosensor comprising:

a container for biological sample subject to screening, said container having

a first electrode in contact with said sample,

a second electrode below said sample,

means responsive to the change of conduction between said electrodes,

a membrane below said sample, said membrane located between said electrodes, and having ion channels therein attached to first detector moieties at an upper surface to which the antibodies or antigens of interest specifically bind, the binding of the antibodies or antigens to the first detector moieties causing a change in the ability of ions to pass through the membrane via the ion channels;

and;

(2) measuring change in conductance between the two electrodes.

In a preferred form of this apect of the present invention the membrane further comprises membrane spanning components attached to second detector moieties to which the antibodies or antigens of interest specifically bind, wherein the binding of the antibodies or antigens of interest to the first and second detector moieties crosslinks the ion channels to the membrane spanning components.

It is also preferred that the first and second detector moieties are the same.

In one form of the invention it is the presence of antibodies of interest in the biological sample that are to be detected and in other embodiments these are antibodies directed against red blood cell antigens or antigens from a pathogen preferrably from a pathogen selected from the group consisting of hepatitis and HIV.

In another form of the invention it is the presence of antigens of interest in the biological sample are detected. These antigens are preferably red blood cell antigens or antigens from a pathogen preferably from a pathogen selected from the group consisting of hepatitis and HIV.

In yet another preferred embodiment the first and second detector moieties are red blood cells or fragments thereof carrying predetermined antigens.

In a fourth aspect the present invention consists in a method of screening for the presence of antibodies in a biological sample directed against distinct epitopic sites on the same antigen, the method comprising the steps of:

(1) optionally adding the antigen to the biological sample;

(2) introducing the biological sample into a biosensor, the biosensor comprising:

a container for biological sample subject to screening, said container having

a first electrode in contact with said sample,

a second electrode below said sample,

means responsive to the change of conduction between said electrodes,

a membrane below said sample, said membrane located between said electrodes, and having ion channels therein attached to first moieties at an upper surface which bind antibodies;

and;

(3) measuring change in conductance between the two electrodes.

In a preferred embodiment of this aspect of the present invention the membrane further comprises membrane spanning components attached to second moieties which bind antibodies.

It is also preferred that the first and second detector moieties are the same and are preferably anti-Fc antibodies or Fc binding fragments thereof.

As will be understood by those skilled in this field it is also possible to include further detector moieties attached directly to membrane components other than the membrane spanning components. Further, as will be readily apparent it is possible to use a range of differing detector moieties attached to the ion channels and/or the membrane spanning components. This may be of benefit where it is desired that a range of antibodies or antigens are detected.

As will be apparent in one form of the invention the cell comprises an ion channel sensor in which the ionophore and membrane spanning lipids

(MSL's) present molecules capable of binding to epitopic sites on a red blood cells such that the arrival of the blood cell at the membrane crosslinks the ionophore and MSL and affects the ionic current through the membrane or implements one of the other mechanisms for affecting ionic current through the membrane in a measurable manner. The mixture of blood products is introduced into the container above a thin layer of preferable glass or plastic percolation beads through which the mixture of samples must pass to reach the membrane. The percolation layer unlike the prior art is capable of having a thickness substantially less than several centimetres. The speed of the process is accomplished by the rapid diffusion of the blood sample though the thin percolation bead layer when no coagulation occurs. The discrimination of the device is accomplished by the different abilities coagulated and uncoagulated blood cells to pass through the filter layer to the membrane.

The preferred molecules capable of binding to epitopic sites on a red blood cell are antibodies that selectively bind to glycophorin molecules that present on the surface of the red blood cell.

In a further embodiment of the present invention the presence of antibodies to multiple epitopes on a large antigen such as a viral particle, a protein fragment from a cell or a virus or an intact cell is detected by anti-Fc Fab' being attached to both the ion channel and the MSL (membrane spanning lipid) within the sensor membrane. The unbound Ab having been selectively scavenged by an excess of anti-Fc antibodies distributed over the electrode surface.

The Abs bound to the large Ag is separated from the unbound free Abs via a bead through which the unbound Abs can pass more readily than the Ab-Ag complex. Through spatially distributing electrodes away from the point of sample addition the percolation of the free Ab to the electrodes may be separated from the percolation of the large Ag-Ab complex.

In another embodiment of the present invention, the presence of Abs to a variety of epitopic sites on large protein Ags is detected by tethering the large Ag or Ag fragment to both the MSL and to the mobile outer layer ion channels, such that with the addition of patient serum Ab's to be detected, Abs reactive with the epitopic sites on the Ags cause the Ags to cross link preventing the diffusion of the outer membrane layer of the gA, reducing its admittance, thereby reporting on the binding.

A further embodiment of the present invention concerns the determination of the species of an Ab, for example as to whether it's IgG or IgM. In the present invention the Abs are required to pass through a bead bed labelled with either IgG or IgM molecules preventing the passage of bound antibody to the electrode surface and to the reporter antigens.

The invention further provides for the determination of the coagulation state of a population of erythrocytes. The present invention used an array of small diameter (typically 1-100 μ, preferably 10-90 μ) gold electrodes bearing a biosensor membrane in which the Fab' recognition element was typically anti-glycophorin. Each electrode is independently addressable using a multiplexed array. The addition of a sample of uncoagulated erythrocytes results in a capture and measurement of a binding event at a distribution of the small electrodes reflecting the random dispersal of the erythrocytes in the sample. The addition of a sample of coagulated erythrocytes would result in the measurement of binding at electrodes that were non-randomly distributed over the array. The difference in the pattern of response to the presence of the erythrocytes across the array reporting the state of aggregation of the erythrocytes. A further embodiment of this approach would be to employ the array to determine cells of differing size and surface antigens. The utility in the latter case being the ability to sort the population of different cell species within the sample.

A further embodiment is to use these arrays to may antigenic sites over the surface of individual cells.

Brief Description of the Drawings

Figure 1 is schematic representation of the preferred embodiment of the ICS of the present invention.

Figure 2 is a schematic representation of the elements of the ICS used to detect the presence of antibodies to multiple epitopes on a large antigen.

Figure 3 is a schematic representation of the elements of the ICS used to detect the presence of Abs to a variety of epitopic sites on large protein Ags.

Figure 4 is a schematic representation of the array of gold electrodes in a multiplexed array.

Detailed Description of a Preferred Embodiment

Referring to Figure 1, a biosensor cell 1 is depicted for detecting the coagulation of blood in an analyte. The cell is has a volume 3 for holding a sample of blood products 5 subject to testing. A first electrode 7 is in contact with the sample. A second electrode 9, is below the volume 3, and below a membrane 11. The electrodes are connected to a voltmeter or impedance measuring device 13 for responding to the change of conduction between said electrodes.

The membrane 11 is preferably a amphiphilic bilayer membrane. It comprises membrane spanning lipids 15, half membrane spanning lipids 17 and ionophores 19 below the volume 3. The ionophores are preferably dimers such as gramicidin A which comprises two monomers that organise into the corresponding layers of the bilayer membrane. The membrane is located between the electrodes so that its electrical impedance properties can be measured by the electrodes. Although so described any arrangement of electrodes that would permit the determination of the ionic conductivity is within the scope of the invention.

The membrane has its ion channels 19 covalently attached to detector molecules 21 at an upper surface that bind to epitopic sites 23 on blood components. The ion channels have a conductivity to ions that changes upon the binding of the molecules 23 to the blood components.

A key component of the invention is a filter layer between the membrane 11 and the volume 3. The blood components if uncoagulated will pass through the filter and contact the membrane.

The membrane preferably also comprises membrane spanning lipids 25 attached to the same type molecules 21 that bind to sites on blood components or to a different type. As a result of the binding of two sites on a single large blood cell, the presence of a blood component at the surface of the membrane crosslinks the upper portion of an ion channels to a membrane spanning species of molecules.

Preferably the molecules that binds to the blood cells are an antibody to glycoprotein, a fragment of a derivative thereof.

Various strategies known previously may be used to implement varying the conductivity if the ionophore. These strategies include local disruption gating, extended disruption gating, vertical disruption gating, and extended displacement gating. Where required by the strategy the membrane spanning components 15 may be tethered by linking and spacing molecules

to the second electrode 9. In addition the linking and spacing molecules may be those known in the prior art to provide an ionic reservoir region.

Where the present invention has been described in terms of the use of blood cells, it should be apparent that the methods and apparatus of the present invention are susceptible of adaptation to other the determination of epitopic sites on other large molecules or cell types.

Referring to Figure 2, antibodies to multiple epitopes on a large antigen such as a viral particle, a protein fragment from a cell or a virus or an intact cell are depicted. The sample is introduced as one side of the ICS and allowed to percolate laterally. The presence of one antibody is detected by anti-Fc Fab' being attached to both the ion channel and the MSL (membrane spanning lipid) within the sensor membrane. The unbound Ab is scavenged by an excess of anti-Fc antibodies extending along the electrode surface. By percolation, or diffusion through a bead bed, the Abs bound to the large Ag are spatially separated from the unbound free Abs. As depicted in the figure, the unbound Abs pass through the bead bed more readily than the Ab-Ag complex. In this way the bound Abs reach a portion of the membrane uncluttered by the unbound Abs. Since the unbound Abs do not cross link the ionophores of the membrane, the change in ion current though the membrane in a cell such as that shown in Figure 1 is due entirely to the presence of the bound Ab.

In this matter tests may be made for the presence of the antibody to Hepatitis A virus, Hepatitis B core viral proteins, Hepatitis C virus, HIV virus types 1 and 2, HTLV types 1 and 2, p24 antigen, and Hepatitis B surface antigen.

Figure 3 depicts the procedures for detecting the presence of Abs to epitopic sites on large protein Ags. This method is appropriate whenever the Ag fragments are characterised by a unique structure.

Figure 4 is a perspective view of an array of cells for determining the coagulation states of a population of erythrocytes. The individual cells are constructed as in Figure 1, except that their upper and lower electrodes are connected respectively to the side and bottom edges of the array where they can be polled to determine the conductivity of the individual cells using standard multiplexing electronics. In this manner, the presence of binding on a scale of a tens of microns is achieved.

DETECTION OF ANTI-D BLOOD TYPE ANTIGEN

METHOD

1st membrane layer: 9.3nM Gayy (Linker Gramicidin B) (Fig. 5)
5.5nM MSLXXXXB (MSL-C) (Fig. 6)
1.1uM MSLOH (MSL-D) (Fig. 5)
37uM MAAD (mercaptoacetic acid disulfide)
75uM DLP (Linker A) (Fig. 7)

2nd layer: 10mM (DPEPC:GDPE=7:3) : Ga5XB (Biotinylated Gramicidin E; Fig. 8) = 66,667:1

Membranes and electrodes were prepared as described in PCT/AU95/00763. Briefly, electrodes with freshly evaporated gold (1000Å) on chrome adhesion layer (200Å on glass microscope slides) were dipped into an ethanolic solution of the above components, rinsed with ethanol, then stored at 4°C under ethanol until used for impedance measurements. The slide was clamped into a block containing teflon coated wells which defined the area of the working electrode as approximately 16mm^2.

The 2nd layer was added from an ethanolic solution then phosphate buffered saline (PBS) was added, and the electrode washed 4 times. Streptavidin was added to each well (5µl 0.1mg/ml in PBS) and incubated 5 minutes before washing out excess unbound streptavidin with PBS. Fab' fragments biotinylated at the free thiol group (Fab' were prepared from IgG antibodies raised against human anti-D blood type antigen) were then added (5µl 0.05mg/ml in PBS) and rinsed with PBS after 10 minutes. Reagent red blood cells (3% cell suspension from the "Surgiscreen" kit by Ortho Diagnostic Systems Inc) with and without the anti-D blood type antigen, were added to different electrodes. A change in impedance was observed only in the presence of red cells which possessed the anti-D blood type antigen.

While there have been shown and described and pointed out the fundamental novel features of the invention as applied to preferred embodiments thereof, it will be understood that various omissions and substitutions and changes in the form and details of the device illustrated

and in its operation may be made by those skilled in the art without departing from the spirit of the invention.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

CLAIMS:

1. A method of screening for the presence of antibodies or antigens of interest in a biological sample, the method comprising the steps of:

(1) introducing the biological sample into a biosensor, the biosensor comprising:

a container for biological sample subject to screening, said container having

a first electrode in contact with said sample,

a second electrode below said sample,

means responsive to the change of conduction between said electrodes,

a membrane below said sample, said membrane located between said electrodes, and having ion channels therein attached to first detector moieties at an upper surface to which the antibodies or antigens of interest specifically bind, the binding of the antibodies or antigens to the first detector moieties causing a change in the ability of ions to pass through the membrane via the ion channels;

and;

(2) measuring change in conductance between the two electrodes.

2. A method as claimed in claim 1 wherein said membrane further comprises membrane spanning components attached to second detector moieties to which the antibodies or antigens of interest specifically bind, wherein the binding of the antibodies or antigens of interest to the first and second detector moieties crosslinks the ion channels to the membrane spanning components.

3. A method as claimed in claim 2 wherein said first and second detector moieties are the same.

4. A method as claimed in any one of claims 1 to 3 in which the presence of antibodies of interest in the biological sample are detected.

5. A method as claimed in claim 4 in which the antibodies of interest are antibodies directed against red blood cell antigens.

6. A method as claimed in claim 4 in which the antibodies of interest are antibodies directed against a pathogen.

7. A method as claimed in claim 6 in which the pathogen is selected from the group consisting of hepatitis and HIV.

8. A method as claimed in any one of claims 1 to 3 in which the presence of antigens of interest in the biological sample are detected.

9. A method as claimed in claim 4 in which the antigens of interest are red blood cell antigens.

10. A method as claimed in claim 4 in which the antigens are antigens of a pathogen.

11. A method as claimed in claim 6 in which the pathogen is selected from the group consisting of hepatitis and HIV.

12. A method as claimed in claim 5 in which the first and second detector moieties are red blood cells or fragments thereof carrying predetermined antigens.

13. A method of screening for the presence of antibodies in a biological sample directed against distinct epitopic sites on the same antigen, the method comprising the steps of:

(1) optionally adding the antigen to the biological sample;

(2) introducing the biological sample into a biosensor, the biosensor comprising:

a container for biological sample subject to screening, said container having

a first electrode in contact with said sample,

a second electrode below said sample,

means responsive to the change of conduction between said electrodes,

a membrane below said sample, said membrane located between said electrodes, and having ion channels therein attached to first moieties at an upper surface which bind antibodies;

and;

(3) measuring change in conductance between the two electrodes.

14. A method as claimed in claim 13 wherein said membrane further comprises membrane spanning components attached to second moieties which bind antibodies.

15. A method as claimed in claim 14 wherein said first and second detector moieties are the same.

16. A method as claimed in any one of claims 13 to 15 in which the first and second moieties are anti-Fc antibodies or Fc binding fragments thereof.

17. A method as claimed in any one of claims 1 to 16 in which the membrane further includes additional detector moieties attached to the membrane.

18. A biosensor for detecting the coagulation of blood in an analyte comprising

a container for a blood sample subject to testing, said container having

a volume for holding a sample of blood products subject to testing,

a first electrode in contact with said sample,

a second electrode below said volume,

means responsive to the change of conduction between said electrodes,

a membrane below said volume, said membrane located between said electrodes, and having ion channels therein covalently attached to detector molecules at an upper surface that bind to epitopic sites on blood components, said ion channels having a conductivity to ions that changes upon the binding of said molecules,

said volume contacting a filter layer between said membrane and said volume, wherein said blood components which pass said filter contact said membrane.

19. The biosensor of claim 18 wherein only uncoagulated blood components pass filter.

20. The biosensor of claim 18 or 19 wherein said membrane further comprises membrane spanning components attached to a second species of molecules that bind to sites on blood components, wherein the presence of a blood component at the surface of the membrane crosslinks the ion channels to said second species of molecules.

21. The biosensor of claim 20 wherein said second species of molecules is the same as said detector molecules.

22. The biosensor of claim 18 wherein the conductivity to ions that changes upon the binding to said molecules is due to local disruption gating.

23. The biosensor of claim 18 wherein the conductivity to ions that changes upon the binding of said molecules is due to extended disruption gating.

24. The biosensor of claim 18 wherein the conductivity to ions that changes upon the binding of said molecules is due to vertical disruption gating.

25. The biosensor of claim 18 wherein the conductivity to ions that changes upon the binding of said molecules is due to extended displacement gating.

26. A method of determining compatibility of two blood samples, the method comprising the steps of:

(1) mixing two blood samples to form a blood mix;

(2) introducing the blood mix into a biosensor as claimed in any one of claims 18 to 25; and

(3) measuring change in conductance between the two electrodes of the biosensor.

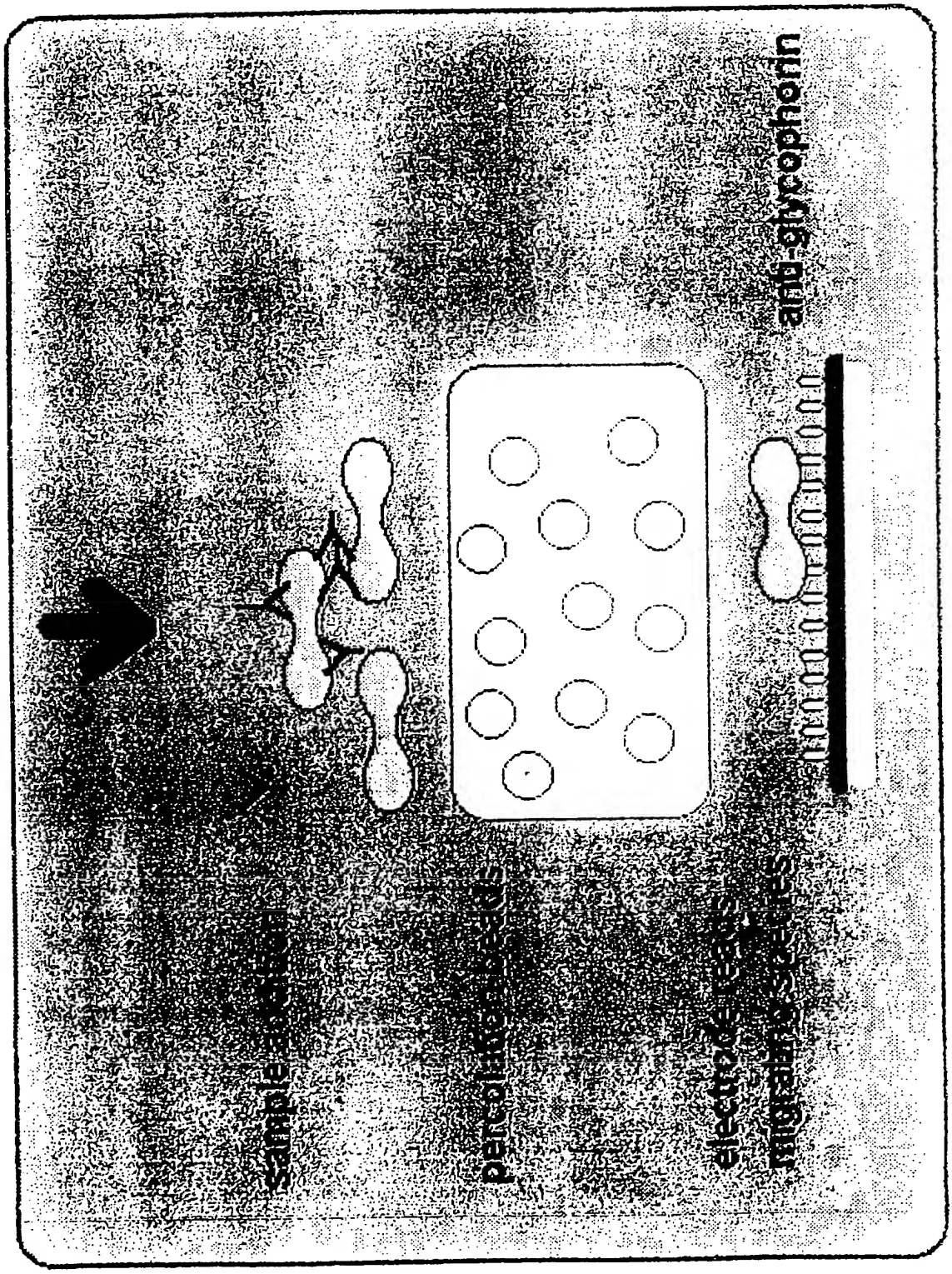


FIGURE 1

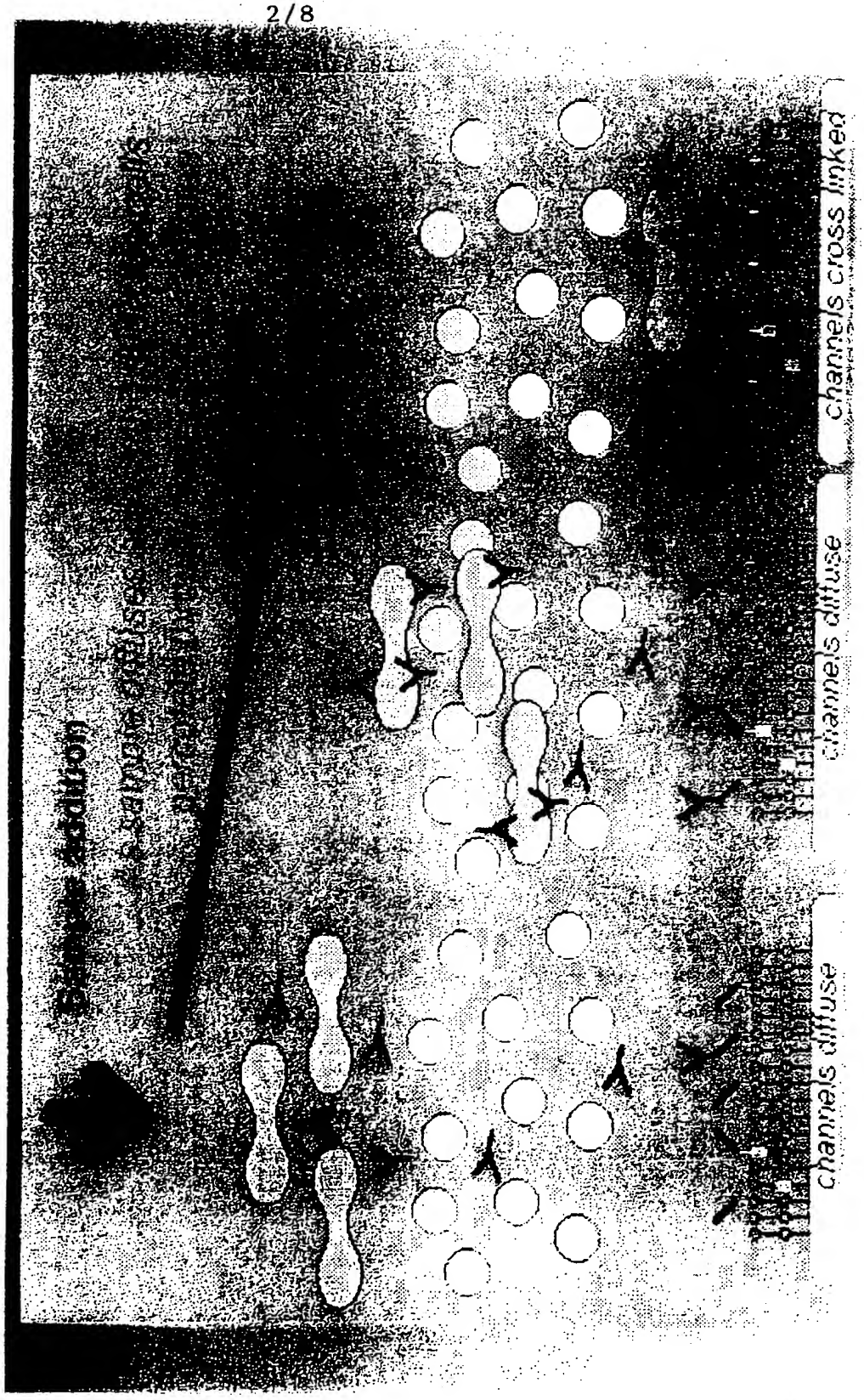


FIGURE 2

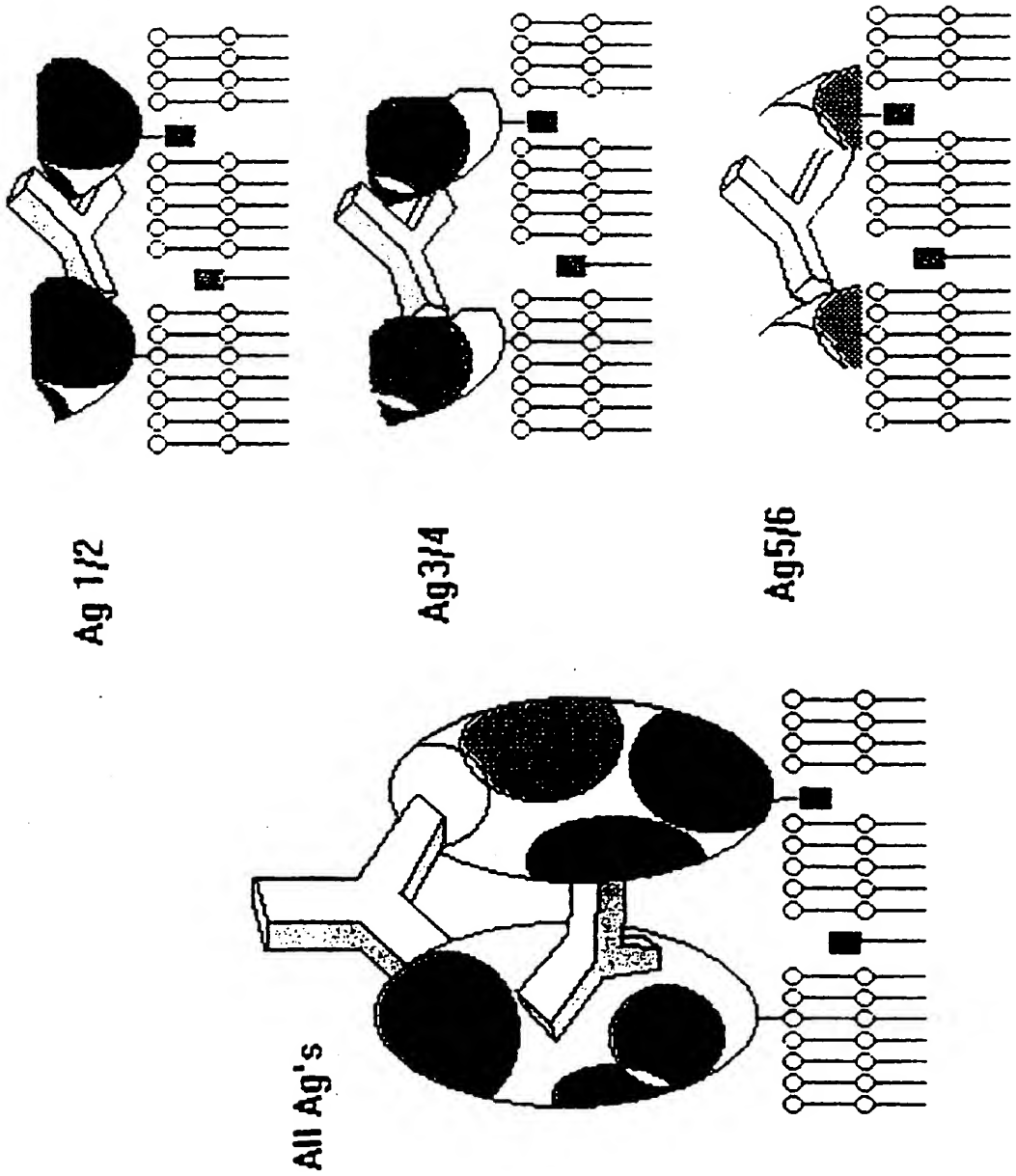


FIGURE 3

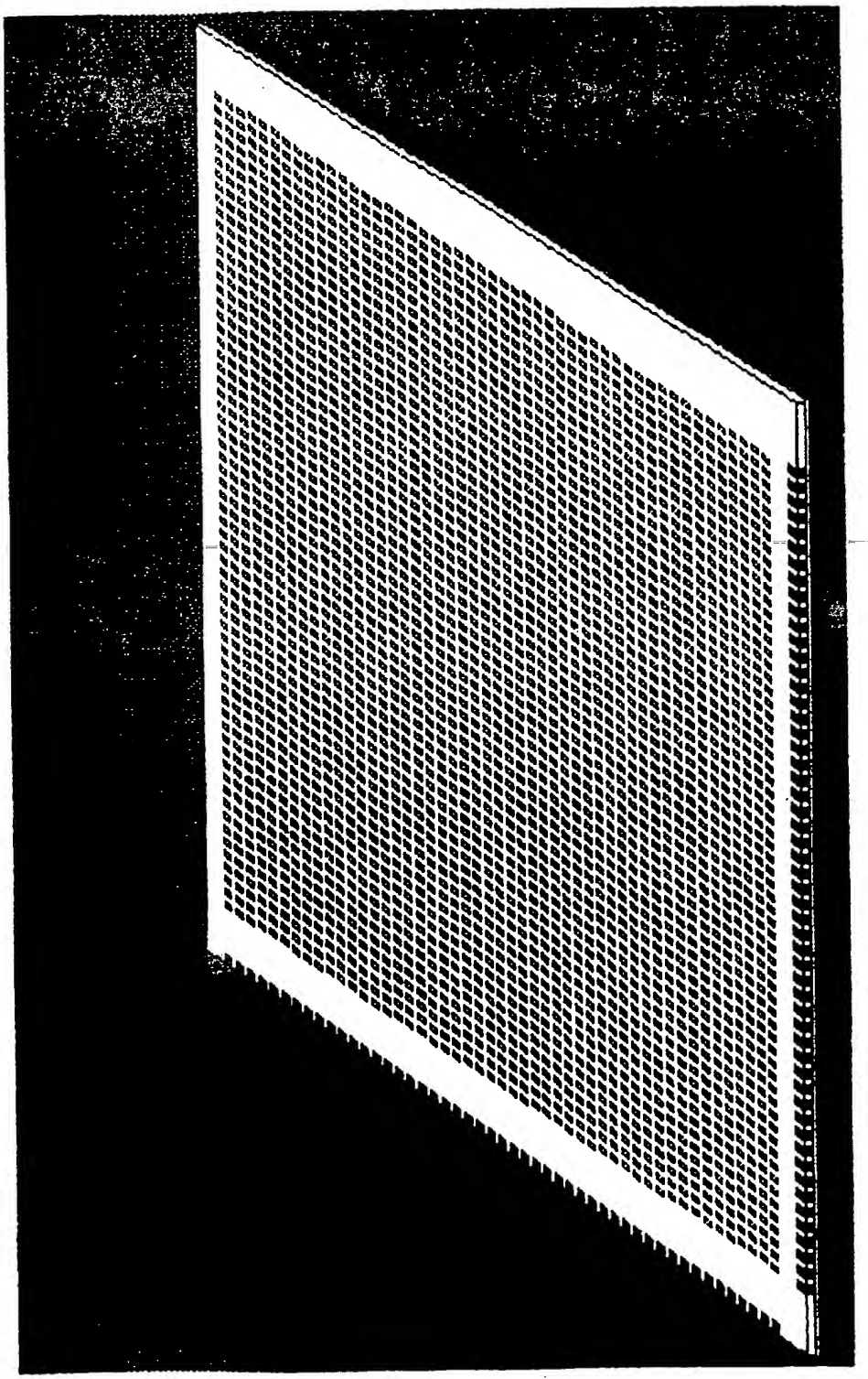
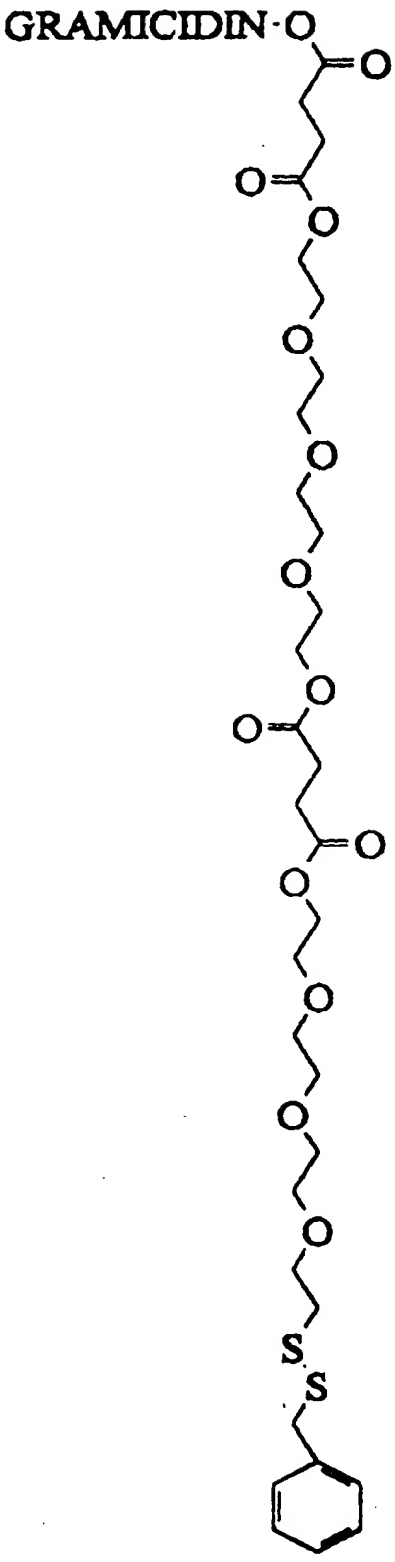
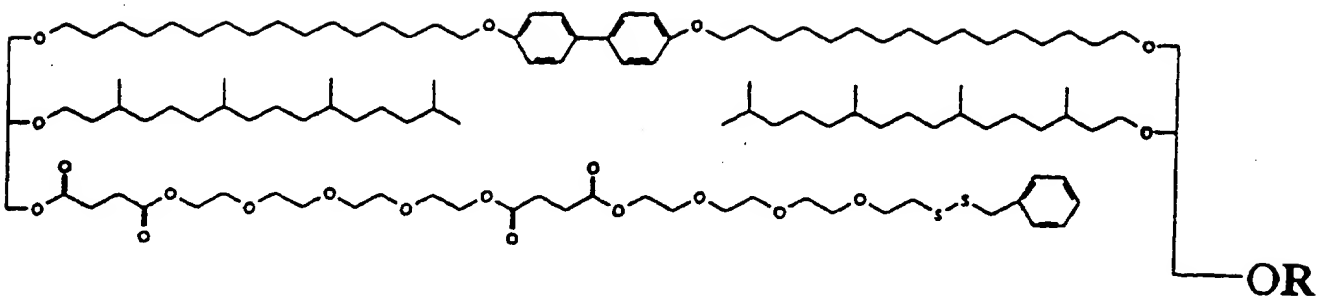


FIGURE 4

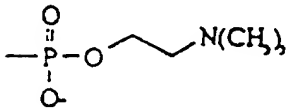


Linker Gramicidin B

FIGURE 5

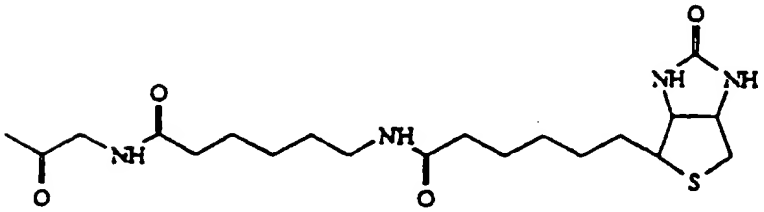


R



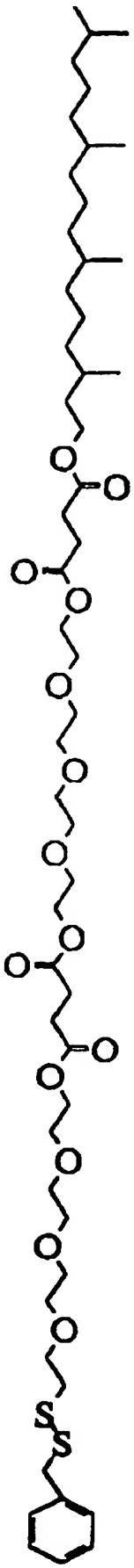
or OH

Membrane Spanning Lipid D



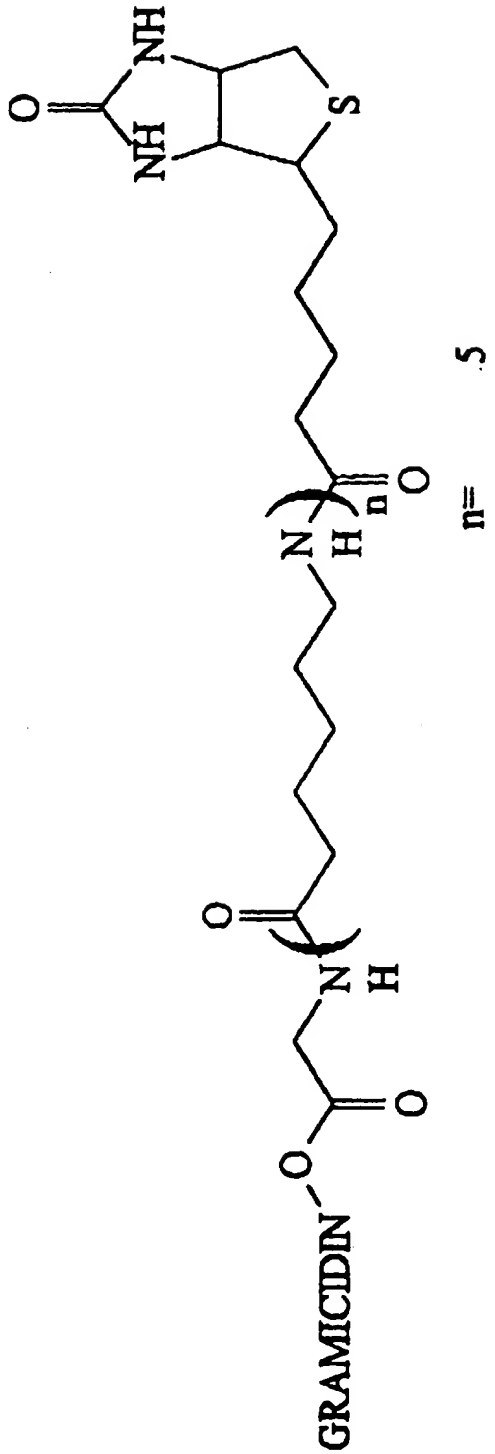
Membrane Spanning Lipid C

FIGURE 6



Linker Lipid A

FIGURE 7



Biotinylated Gramicidin E

FIGURE 8

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 97/00014

A.	CLASSIFICATION OF SUBJECT MATTER
Int Cl[6]:	G01N 27/327, 27/333, 33/50, 33/547, 33/556, 33/571, 33/576, 33/96

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: G01N 33/-; G01N 27/-

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT: Biosensor, Ionophore, Ion Channel

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A, 95/16206 (BIOSYSTEMS TECHNOLOGY (CORP)) 15 June 1995 the whole document.	1-26
X	WO,A, 95/08637 (UNIVERSITY OF WASHINGTON STATE RESEARCH FOUNDATION) 30 March 1995.	1-26
X	AU,A, 69245/91 (YEDA RESEARCH AND DEVELOPMENT CO LTD) 11 July 1991. Page 11 Line 14-Page 13 Line 24	1-26

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 April 1997	1 May 1997 (01.05.97)

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 97/00014

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB,A, 2195450 (UNITED KINGDOM ATOMIC ENERGY AUTHORITY) 7 April 1988. Lines 43-108.	1-26
X	AU,A, 40787/89 (AUSTRALIAN MEMBRANE AND BIOTECHNOLOGY RESEARCH INSTITUTE LTD) 23 March 1990. Page 4 Line 34-Page 6 Line 18.	1-26
X	AU,A, 21279/89 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION) 1 March 1989. Page 2 Lines 5-22, Page 5 Lines 6-32, Page 7 Line 1 to Page 14 Line 31, Examples 5, 7-9.	1-26
X	US,A, 5368712 (SYNPORIN TECHNOLOGIES, INC) 29 November 1994. Column 4 Line 64 to Column 5 Line 20.	1-26
A	WO,A, 87/00168 (WILLIS, J P) 15 January 1987. The whole document.	1-26
A	EP,A, 342382 (GENERAL ELECTRIC COMPANY) 23 November 1989. Column 4 Line 39-Column 6 Line 43.	1-26
A	US,A, 4713347 (SENSOR DIAGNOSTICS, INC) 15 December 1987. Column 7 Line 7-40, Claims 1-85.	1-26
P,X	WO,A, 96/12957 (PITTNER, F. & SCHALKHAMMER, T.) 2 May 1996. The whole document.	1-26
P,X	AU,A, 38643/95 (AUSTRALIAN MEMBRANE AND BIOTECHNOLOGY RESEARCH INSTITUTE et al.) 6 June 1996. The whole document.	1-26
P,A	AU,A, 56403/96 (AUSTRALIAN MEMBRANE AND BIOTECHNOLOGY RESEARCH INSTITUTE et al.) 29 November 1996. The whole document.	1-26
X	AU,A, 65327/97 (AUSTRALIAN MEMBRANE AND BIOTECHNOLOGY RESEARCH INSTITUTE et al.) 8 November 1994.	1-26
X	AU,A, 56188/94 (AUSTRALIAN MEMBRANE AND BIOTECHNOLOGY RESEARCH INSTITUTE et al.) 22 June 1994.	1-26
X	AU,A, 51444/93 (AUSTRALIAN MEMBRANE AND BIOTECHNOLOGY RESEARCH I NSTITUTE et al.) 26 April 1994. Page 5 Lines 21-28, Page 16 Lines 5-26.	1-26

INTERNATIONAL SEARCH REPORT

AUSTRALIAN PATENT OFFICE
SEARCH REPORT

Application No.
AU 97/00014

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AU,A, 50334/90 (AUSTRALIAN MEMBRANE AND BIOTECHNOLOGY RESEARCH INSTITUTE et al.) 24 August 1990. Page 5 Line 33-Page 7 Line 7; Page 10 Lines 3-31; Page 11 Lines 1-7; Page 12 Line 2-Page 15 Line 28, Example 1 - Pages 26-27, Examples 4, 7.	1-26

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.
PCT/AU 97/00014

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Patent Document Cited in Search Report		Patent Family Member					
WO	96/12957	AT	1970/94	EP	734528	WO	9612957
AU	38643/95	AU	38643/95	WO	9615454		
AU	56403/96	AU	56403/96	WO	9636871		
AU	65327/96	AU	65327/94	WO	9424562	EP	695425
		JP	8509807	CA	2161084	JP	8294768
AU	56188/94	AU	56188/94	CA	2150915	EP	672251
		JP	8504943	US	5591647	WO	9412875
AU	51444/93	WO	9407593	EP	670751	JP	8505123
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		US	5443955	WO	9008783		
WO	95/16206	EP	733209	WO	9516206		
AU	69245/91	AU	69245/91	AT	130938	CA	2033776
		DE	69114870	EP	441120	ES	2082867
		IL	93020	JP	6090736	US	5204239
GB	2195450	EP	261887	GB	8622788	GB	8721607
AU	40787/89	AT	136119	CA	1315338	DE	68926118
		EP	432188	JP	4500124	US	5234566
		WO	9002327				
AU	40787/89	AT	136119	CA	1315338	DE	68926118
		EP	432188	JP	4500124	US	5234566
		WO	9002327				
AU	21279/89	NZ	239796				

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INTERNATIONAL SEARCH REPORT
Information on patent family members

International Application No.
PCT/AU 97/00014

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Patent Document Cited in Search Report		Patent Family Member					
US	5368712	US	5516890	US	5368712		
WO	87/00168	AU	60000/86	DK	991/87	EP	228456
		IL	79229	JP	1501618	WO	8700168
		AU	61244/86	CA	1257331	DK	989/87
		EP	230449	IL	79230	JP	63500539
		WO	8700286				
EP	342382	IL	89719	JP	2024548	NO	892020
		US	4920047				
US	4713347	CA	1249026	DK	4384/86	EP	210224
		FI	863679	JP	62502061	NO	863643
		US	4713347	WO	8604147		

END OF ANNEX